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SIMILAR METABOLIC RESPONSE TO ACUTE ETHANOL INTAKE IN PREGNANT AND NON-PREGNANT RATS EITHER FED OR FASTED

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Abstract—1. Plasma ethanol concentration 3 hr after its oral administration (3 g/kg body wt) did not differ in 20 day pregnant rats with virgin controls, and in both groups values were higher when studied after 24 hr fasted than when fed.

2. In fed animals, blood glucose and liver glycogen concentrations were lower in pregnant than in virgin rats, whereas ethanol intake in both groups enhanced blood glucose levels, it reduced liver glycogen content only in virgins. In fetuses, maternal ethanol intake enhanced blood glucose levels.

3. In fasted animals, ethanol intake decreased blood glucose levels in pregnant and virgin animals but did not affect these levels in fetuses.

4. Ethanol intake enhanced β-hydroxybutyrate/acetoacetate ratio similarly in blood of pregnant and virgin rats when either fed or fasted, and it produced the same change in fetuses from fasted mothers.

5. Results indicate that the metabolic response to acute ethanol does not differ between pregnant and non-pregnant animals, and it is proposed that fetuses passively follow the metabolic changes occurring in their mothers after receiving ethanol.

INTRODUCTION

The metabolic response to ethanol differs between fed and fasted subjects and is seen mainly in the change produced in circulating glucose. In the fed state ethanol may produce hyperglycemia (Ammon and Estler, 1967; Tennet, 1941) due to its enhancing effect on hepatic glycogenolysis (Mirone, 1966). In starvation, when liver glycogen stores are low, ethanol causes hypoglycemia (Bleicher *et al.*, 1964; Freinkel *et al.*, 1965a) due to liver gluconeogenesis inhibition secondary to changes in the intracellular redox state caused by ethanol oxidation (Freinkel *et al.*, 1965b; Krebs *et al.*, 1969; Madison *et al.*, 1967). Pregnancy is well known to induce metabolic adaptative changes that are most clearly manifested in the fasting state. The metabolic response to fasting in late pregnancy is greatly accelerated (Freinkel *et al.*, 1970a; Freinkel *et al.*, 1970b) and enhanced gluconeogenesis (Herrera *et al.*, 1969; Metzger *et al.*, 1970), ketone body production (Herrera *et al*, 1969; Bergman and Sellers, 1960) and adipose tissue lipolysis (Knopp *et al.*, 1970; Chaves and Herrera, 1980) are among the most affected parameters in the fasted pregnant rat, compared to virgin controls. Although the effects of ethanol in pregnancy have been extensively investigated, most studies have been focused to determine its negative consequences on fetal development (for recent reviews, Rosett, 1979; Herrera and Llobera, 1981) and no attempts have been made to determine whether the metabolic response to alcohol differ between pregnant and non-pregnant females. In the present study we investigated this point by determining the response to acute ethanol intake on circulating levels of glucose and ketone bodies and liver glycogen concentration in fed and 24 hr fasted 20-day pregnant rats and virgin controls.

MATERIALS AND METHODS

Female Wistar rats were used weighing 160–180 g, fed rat chow *ad libitum* and kept in a temperature (23 ± 1°C) and light controlled (12 hr on–off cycles) room. Half of the animals were mated and the day of pregnancy was determined by the presence of spermatozoids in vaginal smears. They were studied in parallel to virgin controls at day 20 of gestation in the fed state or after 24 hr fast. Ethanol (3 g/kg body wt) as a 40% solution in saline or plain saline was administered by gastric tube without anesthesia and animals were killed by decapitation 3 hr later. Blood was collected from the neck wound into heparinized tubes and the liver was immediately placed in liquid nitrogen. Fetuses were rapidly dissected and decapitated as described above for blood and liver collection.

Aliquots of whole blood were deproteinized with $Ba(OH)_2$–$ZnSO_4$ (Somogyi, 1945) and supernatants were analyzed for glucose (Hugget and Nixon, 1957) β-HO-butyrate and acetoacetate (Williamson *et al.*, 1962). The remaining blood was used for plasma separation and one aliquot was deproteinized with 0.6 M $HClO_4$ containing 40 mM thiourea for ethanol determination by head-space gas chromatography (Perkin Elmer Sigma 3B), using a column packed with Carbowax 1540 15% CNW 80/100. Portions of frozen liver were digested in 40% KOH for glycogen precipitation (Good *et al.*, 1933) which, after acid hydrolysis, was analyzed for glucose (Hugget and Nixon, 1957).

Results are expressed as means +SEM and statistical comparison between the groups was done by the Student's *t*-test.

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Table 1. Plasma ethanol concentration 3 hr after oral administration of 3 g ethanol/kg body weight in fed and 24 hr fasted rats

	Ethanol (mM)		
	Fed	24 hr fasted	*P*
Virgins	8.32 ± 1.28	28.9 ± 5.9	<0.05
20 day pregnants	8.67 ± 2.12	33.2 ± 9.4	<0.05
P'	NS	NS	

Values are means +SEM. *P* corresponds to the significance of the difference between fasted and fed animals and *P'* to that of pregnants vs virgins (NS = not significant; $P > 0.05$). $N = 6$–7 animals/group.

RESULTS

As shown in Table 1, plasma ethanol concentration 3 hr after its oral administration did not differ in 20 day pregnant and virgin rats. Fasting for 24 hr produced a significant and similar increase in plasma ethanol concentration (Table 1) in both pregnant and virgin rats.

Blood glucose levels (Table 2) were significantly lower in pregnant than in virgin animals under basal conditions in both the fed and fasted state and as expected, values in fetuses were always lower than in their respective mothers. Acute ethanol treatment produced a significant increase in blood glucose levels in fed pregnant and virgin rats and their fetuses, whereas the same treatment in fasted rats produced a significant decrease in blood glucose levels in both pregnant and virgin rats and no change in fetuses (Table 2). Liver glycogen concentration (Table 2) in fed animals under basal conditions was significantly lower in pregnant than in virgin rats and it was higher in fetuses than in their mothers. Fasting produced a marked reduction in the liver glycogen concentration in virgin and pregnant rats under basal conditions but did not affect levels in fetuses which remained similar to values when their mothers were fed (Table 2). Ethanol treatment affected liver glycogen concentration only in fed virgin animals, producing a significant decrease, without significantly affecting any of the other groups (Table 2).

Table 2. Concentration of glucose in blood and glycogen in liver 3 hr after oral administration of 3 g ethanol/kg body weight in 20 day pregnant and virgin rats either fed or 24 hr fasted

	Blood glucose (mg/dl)			Liver glycogen (%)		
	Saline (basals)	Ethanol	*P*	Saline (basals)	Ethanol	*P*
Fed animals						
Virgins	100.8 ± 3.7[a]	113.2 ± 3.5	<0.05	4.93 ± 0.5	3.05 ± 0.6	<0.05
Pregnants	79.7 ± 3.0	96.1 ± 2.6	<0.01	2.03 ± 0.2	2.17 ± 0.3	NS
P'	<0.01	<0.01		<0.001	N.S.	
Fetuses	26.0 ± 3.1	39.3 ± 3.0	<0.05	4.26 ± 0.4	3.72 ± 0.4	NS
P''	<0.001	<0.001		<0.001	<0.05	
24 hr fasted						
Virgins	76.6 ± 5.3**	63.3 ± 3.2***	<0.05	0.04 ± 0.0***	0.03 ± 0.0***	NS
Pregnants	48.8 ± 1.9***	42.0 ± 1.5***	<0.05	0.04 ± 0.01***	0.03 ± 0.0***	NS
P'	<0.001	<0.001		N.S.	N.S.	
Fetuses	20.2 ± 2.4	26.2 ± 2.4**	NS	3.23 ± 0.3**	2.96 ± 0.3	NS
P''	<0.001	<0.001		<0.001	<0.001	

[a]Means ± SEM.
P = Probability between ethanol and basal groups.
P' = Probability between pregnants and virgins.
P'' = Probability between fetuses and their mothers.
*Probability between fasted and fed animals (**$P < 0.01$, ***$P < 0.001$).
NS or no asterisk = Differences not statistically significant.
$N = 6$–8 rats per group.

Table 3. Blood concentration of β-HO-butyrate and acetoacetate 3 hr after oral administration of 3 g ethanol/kg body weight in 20 day pregnant and virgin rats either fed or 24 hr fasted

	Blood β-HO-butyrate (μmol/dl)			Blood acetoacetate (μmol/dl)			β-HO-butyrate/acetoacetate ratio		
	Saline (basals)	Ethanol	*P*	Saline (basals)	Ethanol	*P*	Saline (basals)	Ethanol	*P*
Fed animals									
Virgins	12.3 ± 2.6[a]	7.0 ± 1.4	NS	4.5 ± 1.0	1.1 ± 0.4	<0.05	3.6 ± 0.7	9.6 ± 2.5	<0.05
Pregnants	18.5 ± 5.6	14.2 ± 2.4	NS	5.8 ± 1.5	2.3 ± 0.6	<0.05	3.1 ± 1.2	8.7 ± 1.5	<0.05
P'	NS	<0.05		NS	NS		NS	NS	
Fetuses	15.1 ± 4.3	7.4 ± 0.9	NS	5.2 ± 1.1	1.7 ± 0.7	<0.05	3.1 ± 0.8	5.3 ± 1.6	NS
P''	NS	<0.05		NS	NS		NS	NS	
24 hr fasted									
Virgins	203.6 ± 16.8***	255.7 ± 39.7***	<0.05	35.9 ± 5.6***	16.5 ± 3.3***	<0.05	4.8 ± 0.9	9.9 ± 1.7	<0.05
Pregnants	611.5 ± 80.9***	650.2 ± 44.1***	NS	250.3 ± 38.3***	120.1 ± 16.7***	<0.001	2.6 ± 0.4	5.9 ± 0.5	<0.001
P'	<0.001	<0.001		<0.001	<0.001		<0.05	<0.05	
Fetuses	780.4 ± 49.3***	818.4 ± 46.0***	NS	150.8 ± 23.0***	54.1 ± 8.9***	<0.01	4.5 ± 0.4	13.2 ± 1.5**	<0.001
P''	NS	<0.05		<0.05	<0.01		<0.01	<0.001	

[a]Means ± SEM.
P = Probability between ethanol and basal groups.
P' = Probability between pregnants and virgins.
P'' = Probability between fetuses and their mothers.
*Probability between fasted and fed animals **$P < 0.01$; ***$P < 0.001$).
NS or no asterisk = Difference not statistically significant.
$n = 6$–8 rats per group.

As shown in Table 3, blood levels of β-HO-butyrate and acetoacetate did not differ in fed pregnant and virgin rats, under basal conditions and they were similar in fetuses and their respective mothers. Ethanol treatment in fed animals decreased blood levels of β-HO-butyrate slightly but not significantly whereas it produced a significant decrease in acetoacetate levels in all groups. When these values were expressed as the blood β-HO-butyrate/acetoacetate ratio, ethanol was seen to have produced a significant increase in pregnant and virgin rats and a non significant increase in their fetuses, in which values did not differ from those of their mothers (Table 3). Fasting produced a significant increment in the two ketone bodies in pregnant and virgin rats under basal conditions but the change was much greater in the first group (Table 3). In fetuses of basal mothers fasting produced increases in blood levels of both β-HO-butyrate and acetoacetate levels similar to values in their mothers (Table 3). In the fasting state, ethanol treatment produced a slight increase in blood β-HO-butyrate levels that was only significant in virgins (Table 3) but it produced an intense and significant decrement in blood acetoacetate concentration in virgins and in pregnant rats and their fetuses. When these values were expressed as the blood β-HO-butyrate/acetoacetate ratio, it was evident that ethanol produced a significant increment in all three groups (Table 3).

DISCUSSION

Present results show that acute oral ethanol treatment produced similar blood ethanol concentrations and similar metabolic responses in 20 day pregnant and virgin rats independent of their nutritional status, while the ethanol response differed substantially in fed and fasted animals.

The blood ethanol concentration found in pregnant rats did not differ from that of virgins receiving the same dose per unit of body weight, which is in agreement with the known fact that fetal blood and amniotic fluid attain the same ethanol concentration as in mother's blood (Kaufman and Woollam, 1981), indicating that their body distribution is similar to that of nonpregnant subjects and that the ethanol oxidation is not modified by pregnancy in the rat (Kesaniemi, 1974). The increase found in blood ethanol content with fasting may result from both a more rapid gastrointestinal absorption and a slower ethanol metabolism (Smith and Newman, 1959).

Opposite changes found in blood glucose levels after ethanol treatment in fed vs fasted rats are in agreement with previous reports (Potter and Morris, 1980). Hyperglycemia in fed virgin animals after ethanol treatment may be related to enhanced liver glycogenolysis which is known to be directly or indirectly (catecholamine mediated) stimulated by alcohol intake (Perman, 1960; Jauhonen *et al.*, 1975) as it appeared together with reductions of liver glycogen content. In fed pregnant rats this explanation of alcohol hyperglycemia is more difficult to support as liver glycogen concentration did not change after alcohol intake and changes in either glucose production and/or utilization may be responsible for much of the alcohol effect. Reduced basal liver glycogen, secondary to hypoglycemia in fed pregnant rats, may account partially for lack of alcohol effect on liver content, but further studies should be performed to provide a definitive explanation of this mechanism. The increase in blood glucose levels in fetuses of fed alcohol treated pregnant rats may be related more to the changes in blood glucose levels in their mothers than to the direct effects of ethanol on fetal metabolism. Maternal glucose crosses the placenta by facilitated diffusion (Rice *et al.*, 1979) and as the fetus does not perform gluconeogenesis, under normal conditions fetal glycemia passively reflects changes in the maternal condition. Lack of change in fetal liver glycogen coincides with recent findings that, unlike in adults, plasma catecholamine levels in rat fetuses are not modified by acute maternal ethanol intake (Mena, Zorzano and Herrera, unpublished observations). These data indicate that the fetus does not directly respond to the ethanol received through the placenta after acute maternal intake and that its negative consequences are secondary to effects in the mother. This hypothesis is in agreement with the known fact that the fetus has a very slow ethanol oxidation rate (Horiguchi *et al.*, 1971) due to its limited ethanol-oxidizing enzyme activities (Raiha *et al.*, 1967).

In the fasting state, ethanol intake produced hypoglycemia. Our present findings in virgin animals coincide with previous reports (Potter and Morris, 1980; Tramil *et al.*, 1981) and a similar response was found to occur in fasted pregnant rats. In the fasted state, blood glucose levels depend on glucose synthesis which is known to be enhanced in virgin and pregnant animals (Herrera *et al.*, 1969). Ethanol fasting hypoglycemia has been specifically related to an inhibition in liver gluconeogenesis secondary to the change produced in liver redox state (Freinkel *et al.*, 1965b; Madison *et al.*, 1967). Our finding of enhanced blood β-HO-butyrate/acetoacetate ratio after ethanol intake in pregnant and virgin animals is consistent with such a mechanism.

Intensive basal hypoglycemia in fetuses from fasted mothers precludes its further change as a consequence of glucose level decreases after maternal alcohol intake. In this condition fetal ketosis is very high because ketone bodies cross the placenta freely (Scow *et al.*, 1958) and it is known that the fetus may utilize these metabolites as alternative fuels (Shanbaugh *et al.*, 1977) thus conserving glucose. The fact that the blood β-HO-butyrate/acetoacetate ratio in the fetus changes in the same direction as the mother's after alcohol intake seems to be a consequence of the maternal alcohol effects but further studies are required to establish the potential consequences of this marked change in the redox state on fetal metabolism.

In summary, qualitative metabolic effects of acute alcohol intake do not differ in pregnant and virgin rats either fed or fasted. The fetus passively follows the metabolic changes of maternal events but their immediate and prolonged consequences remain to be determined.

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